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ABSTRACTS OF PAPERS AND DISCUSSION

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Antibodies Against Cells

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Cells can be injured by a number of immunological processes. Some of these are indirect (Arthus reaction, tuberculin reaction, generalized anaphylaxis, serum sickness, etc.), but others are more direct and involve antibodies, humoral or "cellular", directed at specific antigens which consist of cell constituents or products.

A survey of recent scientific progress in the study of antibodies to cells reveals that there are numerous factors which have helped to spur progress in this field.

1. It has been possible to develop and to adapt biological methods which can be used to measure the effects of antibodies against cells, often with extremely fine sensitivity. These methods include electron microscopy, isotope localization, cell fractionation methods, and cytochemistry.

2. Cell systems have been utilized to study the effects of antibodies on cells. Some of these effectively avoid the complications of stroma and circulation. They include tissue cultures of normal and neoplastic cells, ascites tumor cells, and semipermeable chambers.

3. There are "practical" problems which have stimulated study of the effects of antibodies against cells. Among these can be mentioned the unusual balance of host and donor cells in irradiated animals, the search for a method of successfully transplanting organs and tissues, the increasing evidence that pathological immune mechanisms play a role in the pathogenesis of diabetes, cirrhosis, refractory anemia, granulocytopenias, thrombocytopenias, thyroiditis, and some aspermias.

4. Cell antigens are becoming better delineated. Methods are at hand to study the histocompatibility of genetic factors, to separate and analyze the antigens of various cell components and to study these antigens in immune systems aided by gel diffusion, immunoelectrophoresis, anaphylaxis after desensitization with interfering antigens, and tumor immunity after tolerance is induced to normal tissues.

In studies in this laboratory during the past few years the phenomena of antibodies against cells have been explored, using the relatively simple system of immune serum produced in the rabbit against ascites tumor cells of the mouse. These studies have indicated that there is a remarkable selective combining of these antibodies with tumor cells, *in vitro* and *in vivo*, and that these antibodies can produce severe cytotoxic changes when they are mixed with complement and the tumor cells simultaneously. Furthermore, marked decrease in ascites tumor and prolonged survival times have been demonstrated *in vivo*. A few cells appear to escape the action of the antibody.

More recently, studies by Fitch in this

laboratory have indicated that the anti-Ehrlich ascites tumor gamma globulin has demonstrable specificity when studied with fluorescence microscopy. Cross reactions with fibrin, with other tissues and other blood elements were not significant. Furthermore, the avidity of the antibody for the cell surface was well demonstrated. Even the few cells which escaped the cytotoxic action of the antibody-complement combination showed definite localization of antibody.

Marvin Stone, in this laboratory, has recently demonstrated another principle which may have far-reaching immunological implications. He has shown that heterologous antitumor gamma globulin can be rendered effective in retarding the growth of subcutaneous tumors, if the animals have been infected previously with Egypt 101 virus. This consistent finding is in contrast to the lack of effect of the antibody alone or the virus alone. Whether other viruses have similar effects on other antibody systems is a question deserving further study.

Other immunocytological principles have been demonstrated recently by work in this department. Rats can be made hypersensitive to soluble antigens (Rowley and associates) and to homologous renal cell extracts (Blozis and Rowley), utilizing pertussis vaccine as an adjuvant. Comparison of this "auto-immunological" renal lesion with the classical antikidney serum lesion using electron microscopy (Spargo) has demonstrated that, although each is associated with a severe nephrotic syndrome, the changes in the fine structure of the glomerulus are quite different.

DISCUSSION

HANS POPPER: I am fascinated by the amount of evidence which has been presented to us in an extremely difficult area, and my question will probably reflect my ignorance of the field more than anything else. In what way is the antibody attacking the tumor cells? Obviously, we are dealing here with large molecular elements. How does the protein attack these cells, does it get into the cell? From what you have told us it appears to me that the endoplasmic reticulum seems to be attacked. Then, I am extremely intrigued with the virus. Would you accept this as a localization in the cell? Is anticellular antibody involved, is there delayed hypersensitivity taking place?

ROBERT W. WISSLER: The antibody, as far as we can tell, is not getting into the cell. It is difficult to make a definite statement, but at least the fluorescent antibody work would indicate that this is the case. The major effect appears to be on the cell membrane, but one cannot say that none gets to the endoplasmic reticulum. I think that the studies that have been done by Dr. Frank Fitch in our department, demonstrate that the primary effect is probably on the cell membrane. I think this is a unique type of cytotoxic reaction. In fact, Drs. Goldberg and Green here in New York tried to duplicate this with a good many other toxins but could not. It is characterized by fluid coming in and protein and potassium leaving the cell rapidly.

As to the role of the virus in the combined reaction, we are just about as puzzled as you are. At first we assumed that the antibodies were simply getting out of the capillaries and in contact with the tumor cells much more readily than otherwise, and Marvin Stone in our laboratory was able to demonstrate that this is not true by a number of clearcut experiments utilizing I^{130}-labelled antibody. There is no increased localization of antibody at the gross level as compared to non-virus infected cells. Whether there is more antibody having brief contact at the cellular level we are not prepared to say. It is not due to dietary factors, i.e. the virus-infected mice consuming less food and therefore growing small tumors. We have controlled this quite well. We are continuing our study of the phenomenon.

PAUL KLEMPERER: In respect to mitochondria, I thought it was a conjecture on your part that localization was on the endoplasmic reticulum. I did not see any pictures of mitochondria.

ROBERT W. WISSLER: I referred to work done here in New York by Drs. Burton Goldberg and Howard Green at New

York University, Department of Pathology. The mitochondria become exceedingly swollen, according to their electron microscopic studies.

RACHMIEL LEVINE: I would like to ask just one question with regard to the relation to diabetes. You mentioned diabetes as a disease in which there may be a possible relationship of immune reaction to etiology or pathogenesis. May we get a little hint on this?

ROBERT W. WISSLER: The best way to get a hint is to ask Dr. Paul Lacy of Washington University to come to speak to you. He has uncovered, I believe, interesting evidence for immune reactions involving insulin in the blood stream of naturally diabetic people. I think you should ask him to discuss this.

HANS POPPER: Regarding the Ehrlich ascites tumor, was it grown in the same species? We had findings very similar to yours, namely, regression of the tumor, which finally turned out to be due to the fact that the tumor was of a protean nature. Is this tumor a genuine tumor of the same species?

ROBERT W. WISSLER: The Ehrlich ascites tumor is, as you probably know, a mouse tumor which will grow in a large number of strains of mice and will occasionally grow in rats. We think it is a suitable tumor for the study of cytotoxic effects of antibodies. Studies which are aimed at the problem of host-tumor relationships in man might utilize induced primary tumors in the animal. Our own studies on this subject are just beginning. We are attempting to use the carcinogen-induced breast carcinoma in the rat to get some information on this subject.

*Histologic Observations of the Arterial Wall During Vasoconstriction**

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Vasospasm or vasconstriction is a well-known property of the arterial system. If one uses a very thin-walled elastic tube as a model, a reasonable physical explanation of vasconstriction is possible. However, arteries with appreciable wall thickness, capable of a wide range of diameter changes, can also undergo vasoconstriction, and this introduces a complex biophysical aspect to the problem. The arterial wall is a highly integrated structure with unique mechanical properties. At any given moment the arterial wall is in a state of physical equilibrium between two opposing forces: 1) blood pressure within the vessel exerting a radial pressure, and 2) the tension or stress in the wall itself. Total tension may be calculated as a function of blood pressure and radius of the vessel lumen or, restated, the force due to radial pressure is equal to the force due to tension. Tension in the arterial wall is due to the stretch of the wall beyond its so-called natural or unstretched circumference (elastic tension) and vasomotor tone due to active contraction of elements in the wall (active tension). The sum of elastic and active tensions gives the total tension in the wall. Briefly stated, the arterial wall is composed of endothelial cells, elastic and collagen fibers, and smooth muscle. Endothelial cells are negligible contributors to total tension but are capable of extreme deformation. Elastic fibers can be extended to more than 60 per cent of their original length. On the other hand, collagen fibers are 400 times more resistant to stretch than elastic fibers. Studies of arterial smooth

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muscle are not complete. Properties of smooth muscle in other sites are not necessarily applicable to arterial smooth muscle. Arterial smooth muscle is capable of producing active tension, independent of stretch, in a very efficient manner. The muscle is arranged in a circular or ring-like structure. The exact relationship of these smooth muscle fibers to the elastic fibers coursing through the muscle layer is not completely elucidated. It would appear that elastic and collagen fibers maintain tension without a considerable expenditure of energy. Smooth muscle changes in tension of the wall require considerable energy, and yet smooth muscle tension remains practically unchanged when stretched by a steady force.

The biophysicist views the constricted artery with many questions. Some are: What is the role of intravascular blood pressure? Does the artery really close? How is a vessel nourished when contracted? What is the nature of the forces involved? What are the histological components of vasoconstriction? Can vasoconstriction be differentiated from medial hypertrophy of the vessel wall? In an attempt to answer some of these questions a series of experiments was performed. Large mongrel dogs were anesthetized and the intestinal mesentery rapidly visualized. The small arteries of the distal arterial arcade with an average outside diameter of 0.15 cm. were used in the experiments. Vasoconstriction was produced in a focal manner by the adminstration of a drop of epinephrine in the mesenteric connective tissue adjacent to an artery. The vessel was observed to develop a focal vasoconstriction, and within fifteen minutes complete cessation of blood flow was evident distal to the constricted area. The vessel was then stapled quickly at constant length to a cardboard holder, and quickly frozen in iso-pentane chilled by liquid nitrogen. The artery was then prepared by a modified freeze substitution method in an osmic acid acetone solution for five days. The vessel was embedded and sectioned in a serial fashion, and reconstructions made. In two experiments flow through the artery was measured using ultrasonic flowmeters while blood pressure was monitored by a capacitance manometer. It would appear from the studies carried out to date that at the point of maximal vasoconstriction there is marked deformation of endothelial cells. These cells become almost columnar in shape and bulge into the lumen of the vessel. A dense red cell-packed mass and protein-rich fluid completely fill the lumen and probably offer marked resistance to further constriction. The internal elastic lamina is under maximal tension, as revealed by a marked increase in its convolutions and occasional splitting in places. The elastic lamina appears to be a discontinuous structure when seen in cross-section, and this may be an indication of severe longitudinal folding. The smooth muscle cells immediately adjacent to the elastic lamina show marked deformation of nuclei trapped by the convoluted elastic lamina. The smooth muscle cells in the middle of the muscular coat show little or no change, while the peripheral muscular layer and the adventitia show no change at all. In the area of proximal dilatation the endothelial cells flatten and the internal elastic lamina becomes more extended. At no time was a collapsed vessel noted as might have been suspected from gross observations. Additional work is necessary before any criteria may be developed which will clearly establish vasoconstriction in a vessel. This study shows the wide range of internal diameter changes that may take place in a given vessel, so that resistance to flow may vary very widely. Future studies in close cooperation with the techniques of biophysics should yield new information applicable to understanding arterial function and structure.

DISCUSSION

H. M. ZIMMERMAN: Is it your opinion that focal constriction will occur to the point where the vessel lumen is obliterated?

BERNARD M. WAGNER: Epinephrine produces focal constriction. If you give it enough time you will see the flow stop. To measure flow we have ultrasonic flowmeters. We can get a continuous recording of flow and can see the flow actually stop in these distal segments. Peterson gets continuous

recordings of diameter changes, pressure and volume flow. All of these data can be correlated with samplings of vessels at different times. Our studies would support Peterson's results in that the lumen is not obliterated by collapse of the walls.

H.M. ZIMMERMAN: Have you ever seen vasoconstriction of vessels produce infarction?

BERNARD M. WAGNER: We have removed the vessel before damage took place. With the local application of epinephrine one can get extreme vascular constriction and can watch the intestine darken and become ischemic. If one injects the epinephrine intra-arterially the effect does not last long. Vasoconstriction occurs, but it disappears within fifteen minutes. We have not really seen infarction of the intestine.

MAX WACHSTEIN: Were the breaks you have described reversible? Have you studied vessels afterwards?

BERNARD M. WAGNER: With the various components in the wall that are active all the time, one just has to manipulate vessels to see changes taking place. In the so-called normal vessels some of these discontinuous segments can also be found. I don't know what this means. Elastic tissue has some interesting thermo-elastic properties. The biophysicists are not too upset that we see these breaks in the elastica. In addition, there is electronmicroscopic evidence to support this observation.

HANS POPPER: You mentioned that there was complete interruption of blood flow. You avoided the term thrombus. Does thrombosis occur and is this attributed to the damage?

BERNARD M. WAGNER: If the vasoconstriction is maintained for a long time a thrombus may develop. The thing that has intrigued me is the extreme changes the smooth muscle cells can undergo.

HANS POPPER: In other words, this is reversible?

BERNARD M. WAGNER: To a point. Some of them appear as though they are being pinched into two parts, and then, if the vessel is relaxed, we have seen no change in the nuclei that we could call damage.

ROBERT W. WISSLER: About a year ago Dr. Fitch studied two carotid arteries, one of which was ligated, and he studied particularly the elastic tissue. He demonstrated changes in both of these in relation to fracturing of the elastic tissue. I don't know if these changes are reversible. I wonder if, at any time, you investigated lipid in these smooth muscle cells. I don't know whether you have followed the work of Dr. McGill. We have been much interested, because he found the first change in the smooth muscle cell just under the intima. This could conceivably be a response to injury. I am intrigued with this. We hope to try to find out whether there is any kind of interrelationship between epinephrine and the changes epinephrine produces.

BERNARD M. WAGNER: The future is unlimited in this field. It is interesting that the elastic fibers seem to begin the tension that a vessel wall has, and maintain the vessel wall against increasing vascular pressure. Our histochemical studies are in progress.